STUDIES ON THE LYSINE DECARBOXYLASE FORMATION OF BACTERIUM CADAVERIS I. ON LIQUID AND SOLID MEDIUM

STUDIES ON THE LYSINE DECARBOXYLASE FORMATION OF *BACTERIUM CADAVERIS*

I. ON LIQUID AND SOLID MEDIUM

By

Fumio KONDO

Department of Animal Husbandry, Faculty of Agriculture, Tohoku University, Sendai, Japan

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Previously, the author employed lysine decarboxylase of *B. cadaveris* for the determination of lysine contained in feeds and pointed out that this determination method will be more applicable for the purpose if the bacterial cells possessing higher enzymatic activity could be obtained with ease and give a good yield (1). From this point of view, the author researched the lysine decarboxylase formation by *B. cadaveris* when grown on liquid and solid media.

In the following experiments, comparison on the yield and activity of acetone dried cells was made on the basis of 500 mg dried cells/*l* medium (2) and 660 μl/mg dried cells/hour as Q_{CO_2} (3).

1. On liquid medium

According to Gale (3), casein tryptic digest was previously used as the nitrogen source for the enrichment of lysine decarboxylase in *B. cadaveris*. In the present experiments, casein tryptic digest was replaced by commercial peptone, "Polypepton".

Experiments

The strain used was *Bacterium cadaveris* (National Collection of Type Cultures ref. No. 6578). After the organism was grown for definite periods at 25°C, the cells were harvested by centrifugation and dried with acetone according to the method indicated by Gale (2). The lysine decarboxylase activity of the acetone dried cell preparation was measured manometrically under the following conditions. The main cup of the Warburg manometer contained 2 *ml* of *M*/5 phosphate buffer of pH 6.0 and 0.5 *ml* of enzyme preparation suspension (10 mg or 5 mg dried cells/*ml* phosphate buffer of pH 6.0) with 0.5 *ml* of *M*/15 L-lysine solution in the side-bulb. The manometers were shaken in a bath at 30°C, and after 20 minutes equilibration the substrate was tipped in from the

side-bulb. The CO_2 evolution was measured at 5 or 10 minutes intervals after tipping. The enzymatic activity expressed as Q_{CO_2} (CO_2 output μl/mg acetone powder/hour) and the yield of enzyme was represented as "unit". One unit of enzyme was defined, according to Gale (3), as the amount which liberates 100 μl CO_2 from L-lysine in 5 minutes at pH 6.0 and 30°C.

Results

1) Effects of tomato juice, KH_2PO_4 and age of culture on cell growth and enzyme formation

The culture media of the following compositions were prepared and subjected to the test.

Medium No. 1 3 per cent peptone and 2 per cent glucose in water
Medium No. 2 3 per cent peptone, 2 per cent glucose and 2 per cent KH_2PO_4 in water
Medium No. 3 3 per cent peptone and 2 per cent glucose in tomato juice
Medium No. 4 3 per cent peptone, 2 per cent glucose and 2 per cent KH_2PO_4 in tomato juice

400 *ml* of each medium was contained in the glass bottle of 500 *ml* content. pH of the media was adjusted to 7.0.

Tomato juice used was prepared as follows : 450 g of tomatoes were minced and boiled with water. After cooling, they were filtered. The filtrate was diluted to 1000 *ml*, sterilized and preserved.

Cultures of both 24 and 48 hours were made by using media No. 2 and No. 4, whereas other media were employed for the culture of 24 hours. The yield and activity of dried cells prepared from these cultures are shown in Table 1.

Table 1. The effect of KH_2PO_4, tomato juice and age of culture

Medium No.	KH_2PO_4 (%)	Tomato juice	Age of culture (hr)	Final pH	Yield of dried cells (mg/*l*)	Activity of dried cells (μl)	Enzyme yield (units/*l*)
1	—	—	24	5.0	250	112.8	23.5
2	2	—	24	6.0	807	18.6	12.5
3	—	used	24	6.0	820	92.9	63.4
2	2	—	48	5.8	787	16.2	10.6
4	2	used	48	6.2	1130	21.2	20.0

As seen from the table, the activities of the dried cells obtained were remarkably lower as compared with Gale's level. Even dried cells obtained from the culture using medium No. 1, which had highest activity throughout the experiment, showed only 1/5 of Gale's level of the enzymatic activity. Except for the results obtained with the medium No. 1, the yields of dried cells

were higher as compared with Gale's level. In the case of 24 hours culture prevented from significant pH-fall by adding KH_2PO_4, the yield of dried cells was considerably increased, but enzymatic activity of them was greatly decreased. In the 48 hours culture using the same medium, the yield and activity of dried cells were not increased over the levels of 24 hours culture.

When tomato juice was used in the medium, the increment of the yield of dried cells was well recognized but the enzymatic activity was greatly lowered.

The glucose and free lysine contents in the media were measured before and after incubation. In the cultured medium No. 1, 63 per cent of glucose and 9.5 per cent of lysine remained, whereas 38 per cent of glucose in the cultured medium No. 3. But in other media, no glucose nor lysine were detected. These facts showed that consumption of glucose and lysine by *B. cadaveris* were different with the media cultured. The lysine in the medium No. 3, which was buffered by KH_2PO_4, was exhaustively consumed. Considering the fact that the presence of lysine in the medium was necessary for the formation of lysine decarboxylase, it seemed likely that incubation of more than 24 hours did not favor the enzyme formation under these conditions.

2) Concentration of peptone supplied and initial pH of the medium

To reveal the effects of peptone concentrations and initial pH of media on the cell growth and enzyme formation, the media containing peptone in 2.4, 3.0, 3.5 and 4.0 per cent, respectively, together with 2 per cent glucose were prepared and their initial pH were adjusted to 7.4 or 6.8. The results obtained are shown in Table 2.

Table 2. Effect of peptone concentration and initial pH

Peptone (%)	Initial pH	Final pH	Yield of dried cells (mg/*l*)	Activity of dried cells (μl)	Enzyme yield (units/*l*)
2.4	7.40	6.30	350	88.1	25.5
3.0	7.40	6.35	300	57.3	14.5
3.5	7.40	6.25	700	134.2	78.5
4.0	7.40	6.44	400	73.6	24.5
2.4	6.80	5.99	650	117.5	63.5
3.0	6.80	6.05	600	90.9	45.5
3.5	6.80	6.01	750	86.5	54.0
4.0	6.80	5.95	700	136.7	79.5

Then, the dried cells which had the highest enzymatic activity were obtained from the culture with 4 per cent peptone medium of the initial pH 6.8, which showed the lowest final pH as compared with the other cultures so far tested. The results in the table were rather suggestive of the fact that peptone concentrations and initial pH of the tested media, both were not factors definitely

influencing the yield and activity of the enzyme preparation.

3) Trained culture

Whether the enzymatic activity was increased by trained culture of *B. cadaveris* in peptone-glucose medium, was tested as follows: *B. cadaveris* was grown for 24 hours at 25°C in the test tubes containing 10 *ml* of 3 per cent peptone-2 per cent glucose medium. After 24 hours, a little portion (0.5 *ml*) of the culture was again inoculated into 10 *ml* of the next medium, which was subjected to the growth of subsequent 24 hours. Such procedure was repeated four times.

After each 24 hours incubation, the contents of the tubes were collected, centrifuged and the cells were harvested. The preparation of acetone dried cells and the measurement of the enzymatic activity were performed as indicated previously. Peptone-glucose medium used in those experiments contained about 1.3 mg/*ml* of free lysine.

Final pH of the culture media was in the range of 5.0–5.2. The enzymatic activity of the dried cells obtained from the first, second and fourth cultures were 232, 237 and 179 μl as Q_{CO_2}, respectively. The activities of the dried cells obtained from the first and second cultures were almost equal, while the activity of dried cells obtained from the fourth culture dropped. From these results, it was mentioned that enzymatic activity of *B. cadaveris* could not be enhanced by such trained culture technique.

4) Effect of pyridoxine and yeast extract

As only a low enzymatic activity was obtained from the cells grown in peptone-glucose medium, further attempt to improve the enzyme formation was made, and pyridoxine or yeast extract were tested on this respect. Then, the following media were prepared.

Medium No. 1 3 per cent peptone and 2 per cent glucose in water
Medium No. 2 Medium No. 1 + 10 mg pyridoxine/100 *ml* medium
Medium No. 3 3 per cent peptone, 2 per cent glucose and 0.5 per cent yeast extract in water

All media were adjusted to pH 7.4. The culture media were inoculated and incubated for 24 hours at 25°C. Then, the cells were separated by centrifugation and the acetone dried cells were prepared as previously mentioned. The results obtained are shown in Table 3.

Table 3. Effect of pyridoxine and yeast extract

Medium No.	Initial pH	Final pH	Yield of dried cells (mg/*l*)	Activity of dried cells (μl)	Enzyme yield (units/*l*)
1	7.40	5.70	900	106	80
2	7.40	5.88	1050	176	154
3	7.40	6.28	1300	141	153

The yield of dried cells obtained from these media exceeded 500 mg/*l*, whereas the enzymatic activity was yet lower than that of Gale's level. A considerable rise of the enzymatic activity was observed when pyridoxine or yeast extract were added to the medium. This effect of pyridoxine was larger than that of yeast extract. Although these facts were consistent with Hino's observation (4) on shaking culture of *B. cadaveris*, the lower content of coenzyme factors in the peptone-glucose medium than casein tryptic digest-glucose medium should not be regarded as a main cause of the low activity of preparation obtained from former medium, since the activity of the preparation from pyridoxine added medium was far lower than Gale's level.

Yeast extract gave a better yield of dried cells than did pyridoxine.

When pyridoxine or yeast extract were added to the medium, the enzyme yields were increased as twice as control.

As a conclusion, when pyridoxine and yeast extract were added to the medium, the yield and enzymatic activity of acetone powder increased, but the increase of enzymatic activity of the perparation were not sufficient.

2. On solid medium

The previous section deals with the enzyme formation of *Bacterium cadaveris* cells grown on the liquid medium. Then, further research was made on the lysine decarboxylase formation of cells grown on the solid medium.

Experiments

From the stock culture of *B. cadaveris*, cells were inoculated to 10 *ml* of 3 per cent peptone-2 per cent glucose medium in a test tube and incubated for 24 hours at 25°C. The culture thus obtained was used as an inoculum. 15 *ml* of sterilized agar medium was poured into Petri dishes and 0.5 *ml* of the inoculum was scattered on the surface of the solid medium in the dish. Then, the bacteria was grown at 25°C for 48 hours. Thirty Petri dishes and 450 *ml* of medium in total were used in one test.

Cells grown on the surface of solid medium were stripped off by spatula and suspended in a small quantity of water. The cell suspension was filtered on paper by suction to eliminate agar blocks contaminated therein. Then the cells were separeted by centrifugation from the filtrate and washings. Cells were washed once with water. From the washed cells, acetone dried preparation was made as indicated previously. The yield and activity of them were measured. Measuring conditions and expression of the results were previously indicated.

Casein tryptic digest was prepared according to Gale, and tomato juice was same as used for the liquid media. The amount of agar-agar added to the medium was 2.3 per cent.

Results

The yield and lysine decarboxylase activity of the dried cells, and enzyme yield from each medium are summarized in Table 4, together with the composition of medium tested.

Table 4. Yield and activity of dried cells resultng from various solid media.

Medium No.	Casein tryptic digest (%)	Peptone (%)	Meat extract (%)	Glucose (%)	KH_2PO_4 (%)	B.T.B.	Tomato juice	Pyridoxine	Yield of dried cells (mg)	Activity of dried cells (μl)	Enzyme yield (units)
Necessity of KH_2PO_4 addition											
1	3	—	—	2	—	—	—	—	920	11	8.4
2	3	—	—	2	—	used	—	—	—	—	—
3	3	—	—	2	2	used	—	—	600	115	57.5
Effect of tomato juice											
4	—	1.0	0.5	2	2	used	—	—	830	63	43.6
5	—	1.5	0.1	2	2	used	—	—	850	64	45.3
6	—	1.5	0.1	2	2	used	used	—	1300	62	67.2
Necessity of glucose addition											
7	—	3	—	—	2	used	—	—	—	—	—
8	—	3	—	—	2	used	used	—	—	—	—
Inhibition of cell growth by B.T.B. and limitation of effect of tomato juice											
9	—	3	—	2	2	used	used	—	1450	62	74.9
10	—	3	—	2	2	—	used	—	1980	63	104.0
11	—	3	—	2	2	—	—	—	1990	65	107.0
Effect of pyridoxine											
12	—	3	—	2	2	—	—	10mg/450*ml*	1950	101	164.4
13	—	3	—	2	2	—	—	20mg/450*ml*	1950	106	222.6
Effect of sterilization procedure											
14	—	3	—	2	2	—	--	20mg/450*ml*	2590	150	323.8

Each yield was the result from 450 *ml* of medium.
When B.T.B. was used, 40 *ml* of 0.2% B.T.B. solution was added per 1 *l* of medium.

1) *Necessity of the addition of KH_2PO_4 to the medium*

Three per cent casein tryptic digest-2 per cent glucose agar medium was at first adjusted to pH 6.6. In this medium, the yield of dried cells was much increased as compared with that in liquid media, whereas enzymatic activity of them was rather lower and NH_3 output was detected showing that the dried cell preparation had lysine deaminase activity. It has been generally accepted that decarboxylase was formed in acid side, while deaminase in the alkaline side of the medium.

Then, the fact that the preparation obtained from medium No. 1 had lysine deaminase activity, suggested strongly that final pH of the medium shifted to the alkaline side.

To detect changes of pH of the medium during cell growth, bromthymol blue (B.T.B.) was added to medium No. 2 as an indicator and initial pH of the medium was adjusted to 6.0 to prevent from alkalization of the medium. The colour of the medium in this culture did not become blue after incubation for 24 hours, showing that pH of the medium was still kept in the acid side, but after 48 hours, the colour of the medium in 20 of 30 Petri dishes became blue, showing that pH of the medium shifted to the alkaline side. In contrast, in the liquid culture, pH of the medium shifted to the acid side during the cell growth by the addition of 2 per cent glucose, and then it was noticed that on the solid medium, alkalization of the medium was not prevented only by the addition of glucose to the medium, because the growth of the cells was more aerobically maintained on the solid medium than on liquid medium. The activity of dried cells obtained from No. 2 was not measured because of this reason.

Then, an attempt was made to improve pH-shift of the medium during cell growth by the enforcement of buffering capacity, and thus 2 per cent of KH_2PO_4 was added to medium No. 3. In this culture, the colour of the medium did not become blue after 48 hours and the culture was well performed in acid *milieu*. In this case, the yield became three folds more than Gale's level, whereas activity was only one-sixth of that. The gas liberated from substrate when this enzyme preparation reacted with the added substrate was composed almost CO_2 alone, and throughly absorbed into KOH solution.

As the addition of 2 per cent of KH_2PO_4 to the medium served to maintain pH of the medium at the acid side, KH_2PO_4 was added to all the media used in the subsequent experiments.

2) Replacement of casein tryptic digest by peptone and effect of tomato juice

The compositions of the medium containing commercial peptone are also shown in Table 4. The yield and activity of dried cells obtained from the media No. 4 and No. 5, in which the added amounts of peptone and meat extract differed from each other, showed almost the same value. As compared with the result of medium No. 3 in which casein tryptic digest was used, the cell yield in this case was considerably increased, while the enzymatic activity was lowered till about half.

Medium No. 6 which had the same composition as of medium No. 5 was dissolved in tomato juice. The enzymatic activity of dried cells obtained from medium No. 6 was almost the same as that of medium No. 5, but the yield was greatly increased. The effect of tomato juice on the yield of dried cells, which was observed on the liquid medium, was also observed in this case.

3) Necessity of glucose in culture medium

For the purpose of simplifying the composition of culture media, effect of the presence of glucose in the medium was tested. In this test, the colour of medium No. 7, and medium No. 8 in which tomato juice was used, became blue after 48 hours incubation. This fact showed that the addition of 2 per cent KH_2PO_4 alone to the medium was not sufficient and the co-addition of glucose was necessary for the prevention of alkalization of medium under such conditions.

4) Inhibition of cell growth by B.T.B. and limitation of effect of tomato juice

The yield of dried cells resulting from 3 per cent peptone and 2 per cent glucose medium (medium No. 9) was slightly increased, but the activity was lower than that of dried cells resulting from casein tryptic digest-glucose medium.

As it became clear from these experiments that 3 per cent peptone-2 per cent glucose-2 per cent KH_2PO_4 medium maintained the acid side during 48 hours incubation, the addition of B. T. B. to the media was eliminated from medium No. 10 to No. 14. As seen from the table, this did not affect the enzymatic activity, while it gave an increase to the yield of dried cells. Then, B. T. B. inhibited the growth of *B. cadaveris* in the medium.

Though tomato juice was not used in medium No. 11, the yield and enzymatic activity of dried cells resulting from it were not different from that of medium No. 10.

From these facts, it was noticed that tomato juice gave favorable effect on the cell growth when B.T.B. was added to the medium, but when B.T.B. was not given to the medium, the effect of tomato juice was not observed. Accordingly, the use of tomato juice was not necessarily favorable.

5) Effect of pyridoxine on the enzymatic activity of dried cells

The enzymatic activity of dried cells obtained from medium No. 3 which consists of casein tryptic digest showed 115 μl as Q_{CO_2}, but that of dried cells resulting from peptone medium showed only about 60 μl as Q_{CO_2}. To improve this point, pyridoxine was added to the medium as in the case of liquid medium previously done.

To the media No. 12 and No. 13, 10 mg and 20 mg of pyridoxine were added to each per 450 *ml* medium. As compared to the results from the medium to which no pyridoxine was added, the yield of dried cells was not increased but the activity was enhanced. The activity of dried cells resulting from medium No. 13 was slightly higher than that of medium No. 12. The effect of pyridoxine addition to medium on the enzymatic activity of dried cells was also observed on the solid medium as was on the liquid medium.

6) Effect of sterilization procedure

Though the media from No. 1 to No. 13 were sterilized after all components were mixed, in the case of medium No. 14, glucose and other components of the medium were separately sterilized and mixed immediately before use. In the culture on the medium prepared, the yield of dried cells was considerably increased and the activity became slightly higher.

From the fact indicated above, it was noticed that the yield of dried cells resulting from the solid medium per unit volume was increased more than that from the liquid medium. In the case of medium No. 14, the yield showed ten folds of Gale's level, but as to the enzymatic activity of dried cells, even the highest value obtained from cells grown in medium No. 14 was only one fourth of Gale's level. This does not entirely agree with Mardashev's results (5) in which he stated that the good yield and high acitvity were obtained from cells grown on the solid medium. However, it is conceivable that if the enzymatic activity can be much more increased by some means, the enzyme yield becomes higher correspondingly to the high yield of dried cells from the solid medium.

Summary

Replacing the casein tryptic digest with commercial peptone, the effects of the composition of medium on the yield, as well as the enzymatic activity of dried cells of *B. cadaveris* were studied. The results obtained were as follows:

1) The enzymatic activity of dried cells resulting from peptone-glucose medium was far lower than that given in Gale's report. Tomato juice did not serve to enrich the activity, but enhanced the yield of dried cells.

2) The concentration of peptone and initial pH of media did not markedly affect the enzyme formation.

3) The trained culture technique did not serve to the enhancement of enzymatic activity of the preparation.

4) Addition of pyridoxine and yeast extract to liquid media affected the yield and activity of the preparation, but not promptly.

5) In solid medium culture, KH_2PO_4, when added to the medium together with glucose, served to prevent from alkalization of media during cell growth.

6) Tamato juice, when in solid medium with B. T. B. (bromthymol blue), reversed the inhibitory effect of the dye and favored the yield of dried cells.

7) Addition of pyridoxine to the solid media favorably affected the activity of the resulting cells, but not promtly.

8) The yield of dried cells resulting from the solid media was apparently increased as compared with that from liquid media.

9) The yield of dried cells was remarkably increased when glucose and other components of medium were separately sterilized and mixed immediately before use.

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References

1) Hatano, T., F. Kondo and H. Itoh (1954). Tohoku J. Agr. Res., **4**, 161.
2) Gale, E. F. (1948). Eiweis-Forschung, Heft **4**, 10.
3) Gale, E. F. and H. M. R. Epps (1944). Biochem. J., **38**, 232.
4) Hino, T. *et al.* (1960). J. Agr. Chem. Soc. of Japan, **34**, 22 (in Japanese).
5) Mardashev, S. R. and R. N. Etingof (1948). Biokhimiya, **13**, 469 (cited from C.A.).